

1





SEQ. ID. NO.:1

GGTATCAGCAAGCCAAACAAAGGCCCCTCATCTAAGCTGTGTTCTTCAGGCCTACCTCCAGCGCCCAGAA TGAGCCTATTGGCCCCCACAGCTCTCAGGAGCAAGAGTGATGTACAGGACATTGTGAGCAAGAAGTGGGT GCTGCAAACTGCATAACCCCCCTCCTACCGGCAAGACACCGAGTGCTCACACAGAGCTTACTCGTAGGAC TTGCCAGCTGGTTAAGACACCCTGCCATTTTCTCTAACAAGCAGGAGTTCAGTTCAGTTCACAGGGAT GGGGTGGGACCAGGATGGCCACTTTGATCACATGGGAGGGGCGTGGTGTTGTGCAGTTAGGAACAAAGTC TCCCCCTATTTAAGTCCAGCGCTCTGTGCTTTAGTTGATCCCTGGTGTCTCGTGTCTTTTGTCTGCTGCTG TCCCGCCACCAGCCCAGCCATGCAGGACCCTGGGTGCTGCTGCTGCTGGGCCTCAGGCTACAGCTGTC CTGACCCTTCCTCTATTCCCTTGGCCAGTGGAGGAGGAGACCCGGCCTTCTGGAACAAGAAGGCAG CCGAGGCCCTGGATGCTGCCAAGAAGCTGCAGCCCATTCAGACATCAGCTAAGAACCTCATCATCTTCCT GGGTGACGGTGAGTGTGAGCGAGGCCTGCCACCCTGGGGCCCTTGTACTCCAAGTACCCAGGGCCACT GGTGGGTACGGACAGGCCTCAGGGTTCAGTCCTGACGAGGTTCTGCTCCTTCAGGAATGGGGGTACCAAC AGTGACAGCCACCAGGATCCTAAAGGGACAGTTGGAAGGTCATCTAGGACCTGAGACACCCCTAGCCATG GACCGCTTCCCATATATGGCTCTGTCCAAGGTGAGTTCTTAGCCACATCTGAAATGACTGATGGGATCCA GGGCAAGGGAGGCAGAGAGGCTCGGGTGAAGAAATAAATGTCTGCTTTGAGCCCAGTTGGGGTGTCTCTG TCCCCAGACATACAGTGTGGACAGACAGGTTCCAGACAGTGCAAGCACGGCCACCGCCTACCTGTGTGGG GTCAAGACCAACTACAAGACCATCGGCTTGAGTGCAGCCGCGAGATTCGACCAGTGCAACACCACATTTG GCAATGAGGTCTTCTCAGTGATGTACCGTGCCAAGAAAGCAGGTGAGTTGGAGCCAGGCTCAGCTATGGG GGGCAAGCCTAGGGGACTGGATGTCTCACCCTGACCTTTGCCGTCTTCAGGAAAATCCGTAGGTGTGGTG ACCACCACAGAGTGCAGCACGCCTCTCCCTCGGGCACATATGTTCACACAGTGAACCGCAATTGGTATG GGGATGCTGACATGCCTGCCTCTGCGCTGCGGGAAGGTTGCAAGGACATTGCTACACAACTCATCTCCAA GGGAGGGAGGGGAGGTCAGGGGGGTCAAGGGGGGAAGGGGTGGTCCCAGGCAAACCTTGTAGACTGAAC TCCCTGGATCTTCTGGGGTCTTTGAGGGCCGGGTAGTTCAGTTCCCACATACCTGGTGAGGAGCTAGGGA CTTGGTGGGGGGGCGAAAATACATGTTTCCTGCTGGAACCCCAGACCCCGAGTATCCAAATGATGCTAATG AGACTGGAACCAGATTGGATGGCAGGAATCTGGTGCAGGAATGGCTGTCAAAGCACCAGGTGACCGACTG CAGAATATTAGTGATACAGTGGAGACCAGGGAAGGGCTTTGAACCTTACCAGTTGCTTATGTCCCTCTAG GGATCCCAGTATGTTTGGAATCGTGAACAACTCATTCAGAAGGCCCAGGATCCGTCAGTGACATACCTCA TGGGTAATGGCCCCACACTTCCTGCACTGGTACACCTCACATGGCAACCACTGATCCTCTGTGTATATAT GTACCGTGACCCCACTGCCAAGCTTGGTGGTCACCAGTATATATTTTGGTTTTGTACCTCAGGCCTCTTT GAGCCTGTAGACACAAAATTTGATATTCAACGAGATCCCCTGATGGACCCATCTCTGAAGGATATGACAG AGACGCCGTGAAAGTGCTAAGCAGGAACCCCAAAGGCTTTTATCTCTTTGTGGAGGGTGAGTCTCCAAG CTCCCATGGAAAGAGGGGACAATGGACAGGGACAGGCTCAAGCTCACTGGCTTCCTGCAGGGGGCCGAAT GAGAGGGCCAGCCAGCTCACTAGTGAACGCGACACTCTGACCATAGTCACTGCTGACCACTCCCATGTCT TCTCCTTTGGTGGCTACACACTTCGAGGGACCTCCATCTTCGGTAGGTTCGGGAACAGTGGCAGGCTGTC TGTGGGGATCTAGCAACGACTGAACCACTGGCCAGGCAAAAGGCGGGGGCTCGTCTAAGCATCATTCTTG ACCTCCATCCTGTATGGCAACGGCCCAGGCTATGTCGGTACAGGGGAAAGACCCAACGTCACCGCCGCTG AAAGCAGTGAGTGCGGTGGGGTGGCTTGCCTGAAGGTCGGGTAGAGGTGACTCAGATCAGAGTCCTCTCC CCCACGCGGGGAGGACGTGGCGATATTCGCGCGTGGCCCGCAGGCGCACTTGGTGCACGGGGTGCAGGA GCAGAACTACATCGCGCACGTCATGGCCTCTGCAGGCTGCCTGGAGCCCTACACCGACTGCGGCTTGGCA CCCCCTGCAGATGAAAGCCAGACCACCACGACAACCCGCCAGACCACCATCACCACCACCACCACCACCA GGCGCTGCTGGCCGGAATGCTGATGCTACTACTAGGGGCTCCTGCGGAGTCCTAAACTCCAGCACATCTA GGCTCCACCCACTAGGTCCCACGCCCTCACCTGGTCCTTCCCTTCCCTGACCTCAGTGCTCCCTGCATTC

TCCCTGCGGGCTCTACCCCAGGATCCTCTCTCTCTTTTCTGCTACTGCCTCATGTCTAGCCCTACCTT GCATTGCAGCTTCCAGGTTCCTCCTACCCAGGCACTCACAAAGGCCAATCACCTCTGAGCTAGCAGCCAG CCTCAGACCCCACAGAGTTACTTCTCCCCAGGCAGCATGACCACCAAGGCCTTGGACCTCCCGGGGCCAAT CCGGACTCTCCTTTTGCCCTCATCCATCAGCCCCTAGAAAAAGATAGGATCCCGCAATAATTTGTGGAGG ACCAAACATGCACCTGCCCATTGGCACTTCCTCCGAGCTTGAATCCATCTTACAGGCTCTGTACCCAGGA CTAAGGCACAAGAGAACACAGAGAGAGGCTGTCTTCCCACTACTCCTCGGTCTAATCTGCTGGCAGGTGG CACATTCAAAACCATCATGGCTCAGCCATACCAACCCACAGAGCGAAGATTCTGAAATCGTTCAGCCCTT TCATGTCTATTGCCCAGCTAGGAGATTCAAAGAGCTGTACCCCACCCCACTCTCAGGTCATCTCAGGTTG CACCTAAATTTCTGAACTGAGAAAAGTCCCTAACTTCCCAGGTCTGCATTCCCCTGGGGAGAGTCAAGTC AATAATAAAAGAATGTATTCAATACAATAGCAATAGTCATTTTCTTTTTCTTCGGCTCAAAACCAGAGCC TAGTGCCTGCTAGGAACGTGCTCTGCCACTGATCCATAGCCCCATATCATCTCCTCCCCTCCCCTCTCCT CCTCCCTCTCCCCTCCTCCTCCTATGACTCTTAGCCCAAGCTGGCCTCAAATTTATGACAGT ${\tt CCACTTGCTACAGTCTCCCAGATGCTGGATTTTAAGTGTGAGCCACACTCCTAGCATCTTAGTAGGACCT}$ TTGCAGAAGGAAAGCCTGAAGTGTCTGGAGCACTGAGTTCAGATGGGGGGAGGGGTAATAGTGGAGCCTCA TGCTAATCCCCCACCCCCAGGCCAGCGATCAGCTGGAAGGTTGCAACGACTGGGTCAGAGAGGGTGGCT GGGACAGAGGATGCAAAGCTGGAGCTGCAAGGAGCTGTGGGAGGAGGAGGAAGAACTTTAAAATCCATGGC AGTGTGGTCACAAGCCTTTGAATAAGAATTCAGGACGTGGTACTTTTTCTATTGCAGGAAATATGCAATC TTTTCCCCTTTTTTCCTGTTTTTTTTTCCATGGGGGGTGGGAATGGGTGTTAGATATAGGAGCTGGTCA GCCAGAGGGGAGATGCAGACCCTAACCATCTCTGACTTGCATTGGAACTTGGTGGAGCACCACCCCAGTA TAGTTCTTGGCCCCTGTCTAACCTGCCCAATGAGGACATTTGAAGGAATTACGTAAAGGTGGATTAAGCT GTGTTTCTCAGTAAGTTTTGCAACACTACAAATTTATCTGTACATTTATGAAGGTACAAAAACACACTTT GCTCCCACTAGTAATATTAGGAAGATTGAATATGCATCCTTATTTGCTAAAATCTTGATTTAACACTGTG AAACATCAATTCGAAATCTTGGCTCTCGGAGTAGTTTATTTCAATTCCGGATTTTAGTGGCTGTCGAGAA

SEQ. ID. NO.:2

AATATGGGAGCTGAATGGAAAAAGGCCATCGTTAACAAAGCTT

MQGPWVLLLLGLRLQLSLSVIPVEEENPAFWNKKAAEALDAAKKLQPIQTSAKNLIIFLGDGMGVPTVTAT RILKGQLEGHLGPETPLAMDRFPYMALSKTYSVDRQVPDSASTATAYLCGVKTNYKTIGLSAAARFDQCNT TFGNEVFSVMYRAKKAGKSVGVVTTTRVQHASPSGTYVHTVNRNWYGDADMPASALREGCKDIATQLISNM DINVILGGGRKYMFPAGTPDPEYPNDANETGTRLDGRNLVQEWLSKHQGSQYVWNREQLIQKAQDPSVTYL MGLFEPVDTKFDIQRDPLMDPSLKDMTETAVKVLSRNPKGFYLFVEGGRIDRGHHLGTAYLALTEAVMFDL AIERASQLTSERDTLTIVTADHSHVFSFGGYTLRGTSIFGLAPLNALDGKPYTSILYGNGPGYVGTGERPN VTAAESSGSSYRRQAAVPVKSETHGGEDVAIFARGPQAHLVHGVQEQNYIAHVMASAGCLEPYTDCGLAPP ADESQTTTTTROTTITTTTTTTTTTTTTTPVHNSARSLGPATAPLALALLAGMLMLLLGAPAES

FIGURE 1B

Fig. 1. Sec. of the sec. of th

DRAFTSMAN

<u>Underlined</u> = deleted in targeting construct

Bold = sequence flanking Neo insert in targeting construct

AAGCTTAATTGGGGGCCAAGTAGACAGCAGGACATTCAGTGTGCCTTGTTTCCTTTGTCT TTTGGCTCCAGGTATCAGCAAGCCAAACAAAGGCCCCTCATCTAAGCTGTTCTTCAGG CCTACCTCCAGCGCCCAGAATGAGCCTATTGGCCCCCACAGCTCTCAGGAGCAAGAGTGA TGTACAGGACATTGTGAGCAAGAAGTGGGTGCTGCAAACTGCATAACCCCCCTCCTACCG GCAAGACACCGAGTGCTCACACAGAGCTTACTCGTAGGACTTGCCAGCTGGTTAAGACAC CAGGATGGCCACTTTGATCACATGGGAGGGGGGTGTTGTGCAGTTAGGAACAAAGTC TCCCCCTATTTAAGTCCAGCGCTCTGTGCTTTAGTTGATCCCTGGTGTCTCGTGTCTTTG TCTGCTGCTGTCCCGCCACCAGCCCAGCCATGCAGGGACCCTGGGTGCTGCTGCTGCTG ${\tt GGCCTCAGGCTACAGCTGTCCCTTAGTGT}\underline{CATTCCAGGTAATGAGGCTCCTTCCAATGAA}$ <u>CACCCATTCCCACCCATGGACCCTTCATGCTGA</u>CCCTTCCTCTGCTATTCCCTTGGCCA GTGGAGGAGAGAACCCGGCCTTCTGGAACAAGAAGGCAGCCGAGGCCCTGGATGCTGCC AAGAAGCTGCAGCCCATTCAGACATCAGCTAAGAACCTCATCATCTTCCTGGGTGACGGT GAGTGTGTGAGCGAGGCCTGCCACCCTGGGGCCCTTGTACTCCAAGTACCCAGGGCCACT GGTGGGTACGGACAGGCCTCAGGGTTCAGTCCTGACGAGGTTCTGCTCCTTCAGGAATGG GGGTACCAACAGTGACAGCCACCAGGATCCTAAAGGGACAGTTGGAAGGTCATCTAGGAC CTGAGACACCCCTAGCCATGGACCGCTTCCCATATATGGCTCTGTCCAAGGTGAGTTCTT **AGCCACATCTGAAATGACTGATGGGATCCAGGGCAAGGGAGGCAGAGAGGCTCGGGTG**AA GAAATAAATGTCTGCTTTGAGCCCAGTTGGGGTGTCTCTGTCCCCAGACATACAGTGTGG ACAGACAGGTTCCAGACAGTGCAAGCACGGCCACCGCCTACCTGTGTGGGGTCAAGACCA ACTACAAGACCATCGGCTTGAGTGCAGCCGCGAGATTCGACCAGTGCAACACCACATTTG GCAATGAGGTCTTCTCAGTGA'IGTACCGTGCCAAGAAAGCAGGTGAGTTGGAGCCAGGCT CAGCTATGGGGGGCAAGCCTAGGGGACTGGATGTCTCACCCTGACCTTTGCCGTCTTCAG GAAAATCCGTAGGTGTGGTGACCACCACCAGAGTGCAGCACGCCTCTCCCTCGGGCACAT GGGAAGGTTGCAAGGACATTGCTACACAACTCATCTCCAACATGGACATTAATGTAAGGA GGGAGGTCAGGGGGTCAAGGGGGGAAGGGGTGGTCCCAGGCAAACCTTGTAGACTGAAC TCCCTGGATCTTCTGGGGTCTTTGAGGGCCGGGTAGTTCAGTTCCCACATACCTGGTGAG CTCTCTGACCACAGGTGATCCTTGGTGGGGGGGGAAAATACATGTTTCCTGCTGGAACCC CAGACCCCGAGTATCCAAATGATGCTAATGAGACTGGAACCAGATTGGATGGCAGGAATC TGGTGCAGGAATGGCTGTCAAAGCACCAGGTGACCGACTGCAGAATATTAGTGATACAGT GGAGACCAGGGAAGGGCTTTGAACCTTACCAGTTGCTTATGTCCCTCTAGGGATCCCAGT ATGTTTGGAATCGTGAACACTCATTCAGAAGGCCCAGGATCCGTCAGTGACATACCTCA TGGGTAATGGCCCCACACTTCCTGCACTGGTACACCTCACATGGCAACCACTGATCCTCT GTGTATATATGTACCGTGACCCCACTGCCAAGCTTGGTGGTCACCAGTATATATTTTGGT TTTGTACCTCAGGCCTCTTTGAGCCTGTAGACACAAAATTTGATATTCAACGAGATCCCC TGATGGACCCATCTCTGAAGGATATGACAGAGACGGCCGTGAAAGTGCTAAGCAGGAACC CCAAAGGCTTTTATCTCTTTGTGGAGGGTGAGTCTCCAAGCTCCCATGGAAAGAGGGGAC AATGGACAGGGACAGGCTCAGCTCACTGGCTTCCTGCAGGGGGCCGAATCGACCGTGGT CACCATCTGGGCACAGCTTATCTGGCGCTGACTGAGGCTGTGATGTTCGACTTAGCCATC GAGAGGCCAGCCAGCTCACTAGTGAACGCGACACTCTGACCATAGTCACTGCTGACCAC GGGAACAGTGGCAGGCTGTCAATTACGTACAGAATACTTCTGAGCCATCGTTTTCTCTGT CTGTAAAATGGACAGAAATGGCACCTGCCTTGTGGGGGATCTAGCAACGACTGAACCACTG GCCAGGCAAAAGGCGGGGGCTCGTCTAAGCATCATTCTTGGCAGGAAAAAGTGTCCCTCT TGTATGGCAACGGCCCAGGCTATGTCGGTACAGGGGAAAGACCCAACGTCACCGCCGCTG AAAGCAGTGAGTGCGGTGGGGTGGCTTGCCTGAAGGTCGGGTAGAGGTGACTCAGATCAG TGTGCCGGTGAAGTCGGAGACCCACGGCGGGGAGGACGTGGCGATATTCGCGCGTGGCCC GCAGGCGCACTTGGTGCACGGGGTGCAGGAGCAGAACTACATCGCGCACGTCATGGCCTC TGCAGGCTGCCTGGAGCCCTACACCGACTGCGGCTTGGCACCCCCTGCAGATGAAAGCCA

GGCGCTGCTGGCCGGAATGCTGATGCTACTACTAGGGGCTCCTGCGGAGTCCTAAACTCC AGCACATCTAGGCTCCACCCACTAGGTCCCACGCCCTCACCTGGTCCTTCCCTTGA TGCTACTGGCCTCATGTCTAGCCCTACCTTGCATTGCAGCTTCCAGGTTCCTCCTACCCA CTTCTCCCCAGGCAGCATGACCACCAAGGCCTTGGACCTCCCGGGGCAATCCGGACTCTC CTTTTGCCCTCATCCATCAGCCCCTAGAAAAAGATAGGATCCCGCAATAATTTGTGGAGG ACCAAACATGCACCTGCCCATTGGCACTTCCTCCGAGCTTGAATCCATCTTACAGGCTCT GTACCCAGGACTAAGGCACAAGAGAACACAGAGAGAGGCTGTCTTCCCACTACTCCTCGG AGTTCTTCCTCGATGTCTCTGGACCAGCTCCACATTCAAAACCATCATGGCTCAGCCATA CCAACCCACAGAGCGAAGATTCTGAAATCGTTCAGCCCTTTCATGTCTATTGCCCAGCTA GGAGATTCAAAGAGCTGTACCCCACCCCACTCTCAGGTCATCTCAGGTTGCACCTAAATT TCTGAACTGAGAAAAGTCCCTAACTTCCCAGGTCTGCATTCCCCTGGGGAGAGTCAAGTC AACCAGAGCCTAGTGCCTGCTAGGAACGTGCTCTGCCACTGATCCATAGCCCCCATATCAT CTCCTCCCCTCCCTCCTCCTCCTCTTCTCCTTCCCCCCTCCTCCTATGACTCTGT AGCCCAAGCTGGCCTCAAATTTATGACAGTCCACTTGCTACAGTCTCCCAGATGCTGGAT TTTAAGTGTGAGCCACACTCCTAGCATCTTAGTAGGACCTTTGCAGAAGGAAAGCCTGAA GTGTCTGGAGCACTGAGTTCAGATGGGGGAGGGGTAATAGTGGAGCCTCAGTTGGAGAGA GACAGCCAGCTGAGCAAGATCCTGAATGAGGTGAAGGCCTGAGCCAACACCACACAGCAG TGCTAATCCCCCACCCCCAGGCCAGCGATCAGCTGGAAGGTTGCAACGACTGGGTCAGA GAGGGTGGCTGGGACAGAGGATGCAAAGCTGGAGCTGCAAGGAGCTGTGGGAGGAGAGGA AGAACTTTAAAATCCATGGCAGTGTGGTCACAAGCCTTTGAATAAGAATTCAGGACGTGG ATGGGGGGTGGGAATGGGTGTTAGATATAGGAGCTGGTCAGCCAGAGGGGAGATGCAGAC CCTAACCATCTCTGACTTGCATTGGAACTTGGTGGAGCACCACCCCAGTATAGTTCTTGG CCCCTGTCTAACCTGCCCAATGAGGACATTTGAAGGAATTACGTAAAGGTGGATTAAGCT GTGTTTCTCAGTAAGTTTTGCAACACTACAAATTTATCTGTACATTTATGAAGGTACAAA AACACACTTTGCTCCCACTAGTAATATTAGGAAGATTGAATATGCATCCTTATTTGCTAA AATCTTGATTTAACACTGTGAAACATCAATTCGAAATCTTGGCTCTCGGAGTAGTTTATT TCAATTCCGGATTTTAGTGGCTGTCGAGAAAATATGGGAGCTGAATGGAAAAAGGCCATC GTTAACAAAGCTT

FIGURE 2B

Gene Sequence Structure *

dq 699

Sequence Deleted

570 bp

Size of genomic: 5293 bp

Targeting Vector* (genomic sequence)
Construct Number: 2109

Arm Length: 5': 1.1 kb 3': 3.3 kb Targeting Vector

- - - - Endogenous Locus

* Not drawn to scale

nic sequence)

5 > CACTTTGATCCATGGGAGGGG
CGTGGTGTTGTGCAGTTAGGAACA
AAGAAGGCAGC
AAGTCTCCCCCTATTTAAGTCCAG
GCTGCCAAGAA
GCACTCTGTGCTTTAGTTGATCCC
TGGTGTCTCGTGTCTTTGTCTGCT
TGGTGTCCCGCCACCAGCC
ATCCAGGCTCTCAGGGC
ATGCAGGCCTCAGGTG
TCCCTTGTAGTTAGTTGTCTGTAC
TCCCTTAGTCTCAGGCTACAGCTG
TCCCTTAGTGTAA

CTCCTTAGTGTCAGGCTACAGCTG
TCCCTTAGTGTAA

CTCCTTAGTGTCAGGCTACAGGCTTCA

(SEQ ID NO: 4)

(SEQ ID NO: 3)

5 -> SAGAACCCGGCCTTCTGGAAC AAGAAGCCGAGGCCTTCTGGAAC AAGAAGCCGAGGCCCTGGAT GCTGCCAAGAAGCTGCAGCCCATT CAGACCTTAGCTAAGAACCTCATC ATCTTCCTGGGCGACGTGAGTGT GTGAGCGAGGCCTGGCCCCTGG GGCCATTGTACTCCAAGTACCCAGGGCCTCAGGGGTTCAA31

3' probe

3. arm

LacZ-Neo Cassette

5' arm

FIGURE 2C